REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	15 January 1999	Final 1 October 1997-30 September 1998

4. TITLE AND SUBTITLE
Children's Hospital of Pittsburgh Histocompatibility Center
Molecular Typing of Alleles at HLA Class I and Class II Loci on Blood Spotted Filter Paper

5. FUNDING NUMBERS
N00014-97-1-1060

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8. PERFORMING ORGANIZATION REPORT NUMBER
335

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10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; distribution is Unlimited.

12. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The activities of the Children's Hospital of Pittsburgh Histocompatibility Center can be classified under three major categories: 1) the repository service; 2) the molecular typing service; and 3) the research activities aimed at improving the technical support on which the previous two services are working. This year in the Repository, where blood samples from volunteers as bone marrow donors are collected from more than 120 recruiting centers nationwide, roughly 150,000 samples were processed. Close to 450,000 vials were stored in three sets of freezers (one sample per donor each freezer), while nearly 130,000 were sent out to other Typing Laboratories. In our laboratories, approximately 10,000 typings were performed with a very high Q.C. standard, as monitored by National Marrow Donor Program. Our Center's research branch synthesized all the oligonucleotides to be used as PCR primers or labelled probes to perform molecular HLA typing. DNA sequencing was also performed when hybridization results were not optimal. Alternatives for storing blood samples (e.g., blood spots on filter paper) or for enhancing typing capabilities (e.g., microchip technology to expedite PCR procedures) were successfully implemented or improved to the point of justifying their future implementation.

14. SUBJECT TERMS

15. NUMBER OF PAGES
7

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-97-1-1060

Total Samples Typed and Results Sent from 10/01/97 to 09/30/98

	Total	Priority 1	Priority 2	Priority 3	No Makes	Navy
1997						
October	1013	119	894	0	0	0
November	851	161	690	0	0	0
December	822	122	700	0	3	0
1998						
January	838	130	708	0	0	0
February	854	263	591	0	1	0
March	1029	348	681	0	2	0
April	820	174	646	0	0	0
May	976	116	860	0	0	0
June	865	300	565	0	0	0
July	805	49	756	0	0	0
August	818	159	659	0	0	0
September	1026	188	838	0	0	0

CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-97-1-1060

Total Samples Received, Stored and Shipped from the Repository from 10/01/97 to 09/30/98

SAMPLES	NUMBER (October1997)	NUMBER (November 1997)	NUMBER (December 1997)
Stored	11,593	13,743	12,523
Shipped (AB - Class I)	4,700	5,800	7,400
Shipped (DR - Class II)	9,394	6,614	8,463
Not stored	87	113	149
Destroyed	0	238	0

SAMPLES	NUMBER (January '98)	NUMBER (February '98)	NUMBER (March '98)
Stored	8,192	7,437	16,264
Shipped (AB - Class I)	3,400	4,300	5,100
Shipped (DR - Class II)	3,578	5,713	5,652
Not stored	47	28	124
Destroyed	1,736	1,941	0

SAMPLES	NUMBER (April '98)	NUMBER (May '98)	NUMBER (June '98)
Stored	14,769	14,028	11,628
Shipped (AB - Class I)	5,250	4,100	5,700
Shipped (DR - Class II)	4,862	4,580	4,140
Not stored	173	99	98
Destroyed	464	0	1,131

SAMPLES	NUMBER (July '98)	NUMBER (August '98)	NUMBER (September '98)
Stored	11,709	10,703	10,601
Shipped (AB - Class I)	5,100	4,300	3,175
Shipped (DR - Class II)	4,031	4,668	5,115
Not stored	101	143	59
Destroyed	1,594	588	2,039

- Stored samples (Multiply number stored x 3 because of triplicate aliquots)
- Shipped samples (Separated by AB and DR samples)
- Not Stored because of insufficient quantity; duplicate tubes; too clotted; broken; wrong anti-coagulant.
- Destroyed because no longer eligible, so that blood sample must be removed from NMDP list.

CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-97-1-1060

Research Completed from 10/01/97 to 09/30/98

Month/ Year:	Oligonucleotides Synthesized:	ABI/PE Sequencing:	Visible Genetics Sequencing:	Research Projects:
Oct '97	31	163 reactions	0*	Began exploring optimal PCR conditions for both HLA-A and HLA-B typing using Visible Genetics Microblaster Sequencer. Continued to expand cell reference lines for cell and DNA bank. Synthesized 1 Taqman Probe.
Nov '97	26	42 reactions	0*	New Rhodamine terminator chemistry validated on sequencer. Rhodamine matrix installed on sequencer. Installation of Visible Genetics Sequencer. Class 1 HLA-A and HLA-B PCR amplification optimized for sequencing conditions.
Dec '97	16	104	14	Performed control samples for Class 1 typing on Visible Genetics sequencer. Continued to optimize amplifications and cycle sequencing for HLA-A and HLA-B.
Jan '98	24	94	5	Began training personnel on Visible Genetics Sequencer for Class 1 typing. Performed a quality control study for Visible Genetics sequencer using 3 unknown typing samples and 3 known typing samples.

Month/ Year:	Oligonucleotides Synthesized:	ABI/PE Sequencing:	Visible Genetics Sequencing:	Research Projects:
Feb '98	62	65	11	Set up data base for Visible Genetics Class 1 typing. Continued validation of Class 1 typing via sequencing. Troubleshooting on Class 1 typing reactions using Visible Genetics sequencer.
Mar '98	31	130	7	Big Dye terminator chemistry validated and installed on sequencer. Optimized cycle sequencing for Big Dye Terminator Kit. Expanded cell line references. Continued troubleshooting on Class 1 typing reactions using Visible Genetics sequencer.
Apr '98	26	88	2	Continued to optimized cycle sequencing for Big Dye Terminator Kit. Evaluated and optimized PCR "clean-up" methods for new sequencing chemistry. Continued to expanded cell line references.
May '98	19	117	3	Installed and updated Visible Genetics sequencer : OpenStep 3.0 software. Synthesized 3 Taqman Probes. Began evaluating DNA sequencing systems using capillary electrophoresis on a computer chip. Continued to expanded cell line references.

Month/ Year:	Oligonucleotides Synthesized:	ABI/PE Sequencing:	Visible Genetics Sequencing:	Research Projects:
Jun '98	43	89	11	Evaluated sequencing methods for chip technology. Developed chip design for DNA sequencing. Initiated orders for chip sequencing. Installed Iomega "Jazz" System tape back-up for the hard drive on the Visible Genetics Sequencer. Synthesized 1 Taqman Probe. Continued to expanded cell line references.

Month/ Year:	Oligonucleotides Synthesized:	ABI/PE Sequencing:	CE Sequencing**:	Research Projects:
July '98	41	124	12	Began instrumental set-up for DNA sequencing via capillary electrophoresis (CE) - computer chip technology. Optimized PCR for CE sequencing
Aug '98	28	86	18	Explored polymers and began initial testing. Continued instrumental set-up for CE chip sequencing runs. Continued optimizing PCR conditions for CE runs.
Sep '98	22	125	10	Continued optimizing PCR conditions for CE runs. Continued to evaluate polymers for CE sequencing. Began literature search on new HPLC techniques for DNA purification.

* Visible Genetics sequencing instrument was not yet purchased at this time.

** Instituted new project utilizing capillary electrophoresis (CE) for DNA sequencing in July 1998.